Claims 6-8 and 10-15 are all the pending claims and Claims 8 and 10-15 are currently under examination. Claims 6-7 are withdrawn from consideration as being drawn to non-elected inventions. By this Amendment, Claims 8, 10, 12 and 14 have been amended, support for which can be found throughout the entire specification and the claims as originally filed. No new matter has been added and consideration and entry of the amended claims is requested.

I. Response to the Examiner's Objections to Claims 10 and 12

A. Claim 10 is objected to by the Examiner because the term *"Acacia"* is misspelled.

Claim 10 has been amended as indicated herein, thereby overcoming the Examiner's objection.

B. Claim 12 is objected to by the Examiner who considers the phrase "representation difference analysis method" grammatically incorrect, and which should instead recite "representation difference analysis".

Claim 12 has been amended as indicated herein, thereby overcoming the Examiner's objection.

II. Response to Rejection of Claims 8 and 14 under 35 U.S.C. §132

A. According to the Examiner, the following phrase in step d) of Claim 8 constitutes new matter: "wherein the primers are designed to hybridize to the mRNA for a plant gene related to the breeding marker".

Claim 8 has been amended as indicated herein, thereby overcoming the Examiner's new matter rejection.

B. According to the Examiner, Claim 14 recites new matter, i.e., the phrase "primers *comprise* the sequences of SEQ ID NOS: 1 and 2".

Claim 14 has been amended as indicated herein, thereby overcoming the Examiner's new matter rejection.

III. Response to Rejection of Claims 8 and 14 under 35 U.S.C. §112, first paragraph

Claims 8 and 14 are rejected by the Examiner under 35 U.S.C. 112, first paragraph, for lack of written description support.

A. The Examiner considers that Claim 8 now recites unsupported subject matter. More particularly, the Examiner rejects the limitation "wherein the primers are designed to hybridize to the mRNA for a plant gene related to the breeding marker". The Examiner does not consider the originally filed specification as providing adequate description of a cDNA amplified by oligonucleotide primers that are designed to hybridize to the mRNA for a plant gene related to the breeding marker nor a method of using such a cDNA.

Applicants submit that the specification demonstrates actual support for the amplification of cDNA plant fragments by the inventive method as shown in Figures 1-3 as well as by the descriptive text for the methodology provided throughout the entirety of the specification. Thus, one of ordinary skill in the art would readily appreciate that the specification supports a method for **identifying or analyzing** DNA from polymorphic forest tree plants. Claim 8 as amended herein, not only meets the written description requirements, but overcomes the Examiner's rejection.

B. The Examiner considers that Claim 14 now recites unsupported subject matter. More particularly, the Examiner rejects the limitation that the "primers

comprise the sequences of SEQ ID NOS: 1 and 2", since according to the Examiner, the specification only teaches primers consisting of the sequence of SEQ ID NOS: 1 and 2.

Claim 14 has been amended as indicated herein, thereby overcoming the Examiner's rejection for lack of written support.

IV. Response to Rejection of Claims 8 and 10-15 under 35 U.S.C §112, second paragraph

Claims 8 and 10-15 are rejected under 35 U.S.C. 112, second paragraph, for indefiniteness.

A.1. The Examiner considers step d) of Claim 8 indefinite for reciting "hybridize to the mRNA for a plant gene related to the breeding marker" in step d.

Claim 8 has been amended as indicated herein, thereby overcoming the Examiner's indefiniteness rejection.

A.2. The Examiner considers step d of claim 8 inconsistent for reciting that the probe is (1) a labeled cDNA of all mRNA obtained from the two individuals and (2) the probe is specific for cDNAs corresponding to plant breeder markers.

Claim 8 has been amended as indicated herein, thereby overcoming the Examiner's indefiniteness rejection.

B. The Examiner considers step f) of claim 8 indefinite since it is unclear whether the genome subtraction occurs between the genomic DNA of the same individual or between genomic DNA of one of the individuals and the DNA fragments of step c.

One skilled in the art would understand that the method involves an inter-individual subtraction step followed by an intra-individual subtraction step. Step f) of

[redacted]

Claim 8 corresponds to the intra-individual subtraction step for the method. Accordingly, step f) of claim 8 as amended herein, thereby overcomes the Examiner's indefiniteness rejection and is now clear and definite.

C. The Examiner considers Claim 8 indefinite since it excludes a positive process step relating back to the preamble. The preamble of claim 8 recites "a method of obtaining a DNA fragment" while the last step of claim 8 is directed to "identifying the DNA fragment" thus it is unclear if the claim is directed to actually "obtaining a fragment" or "identifying a fragment".

The preamble and body of claim 8 have been amended as indicated herein, thereby overcoming the Examiner's indefiniteness rejection.

D. According to the Examiner, Claim 8 is indefinite as it is unclear if the "plant" in step a) is a forest tree plant as stated in the preamble or any plant, which encompasses plants other than forest trees.

Claim 8 has been amended as indicated herein, thereby overcoming the Examiner's indefiniteness rejection.

E. According to the Examiner, Claim 8 is indefinite as it appears to be missing step g.

Claim 8 has been amended as indicated herein, thereby overcoming the Examiner's indefiniteness rejection.

F. According to the Examiner, Claim 11 lacks sufficient antecedent basis for the recitation of "the Acacia" as the term Acacia does not appear previously in the claim or any claim from which claim 11 depends.

Applicants respectfully disagree with the Examiner's position. Claim 11 depends from Claim 10 wherein "Acacia" is first recited, and therefore claim 11 is clear and definite.

V. Response to Rejection of Claims 8, 10-12 and 15 under 35 U.S.C §103

Claims 8, 10-12, and 15 are rejected under 35 U.S.C. 103(a) as being obvious over Phillips in view of Wigler and Frazer et al (Journal of Immunological methods, vol. 207, P 1-12, 1997) and further in view of Pinyopusarerk.

According to the Examiner, it would have been *prima facie* obvious to identify a breeding marker for Acacia auriculiformis using the methods taught by Phillips in view of Wigler and Frazer as Phillips teaches the successful identification of genes with a specific phenotype.

Applicants submit that Claims 8, 10-12, and 15 are nonobvious over the Examiner's cited references for all of the following reasons. Claim 8 (and the dependent claims thereof) is directed to identifying DNA for polymorphic, **sibling forest tree plants** using a method combining an **inter-individual genomic DNA** subtraction step, screening the subtracted genomic DNA with cDNAs derived from mRNA of all test sibling individuals combined, performing **intra-individual subtraction** with genomic DNA from one of the sibling individuals, comparing the DNA fragments to exclude the DNA fragments containing intra-individual polymorphisms and identifying the DNA fragments that are polymorphic between the sibling individuals.

Phillips describes a subtraction strategy between **two cDNA samples**, but this method detects differences only within the tested cDNA. The present method is distinguishable in that inter-individual **genomic DNA** subtraction is performed (step c) of claim 8), thereby allowing the identification and characterization of polymorphisms within an entire genome between two sibling individuals rather than between cDNAs. Phillips does not teach or suggest the use of genomic DNA from two sibling individuals. Additionally, Phillips does not teach or suggest identification

of a breeder marker or subtraction of an entire genome in accomplishing this purpose.

Wigler applies an RDA method to a plant genome to define sequences present in one individual of a family compared to that of an individual from a closely related source (Col. 3, lines 52-60). Even assuming, *arguendo*, that Wigler's disclosure relates to step c) of claim 8, Wigler does not specifically teach subtraction of genomic DNA between siblings from within the same family, i.e., intra-family genomic subtraction. Even if Wigler's disclosure would have been considered by one of skill in the art as being generic to only step c) of claim 8, pursuant to §2144.08 of the MPEP, a claimed species is not rendered obvious by a genus.

Wigler does not provide any motivation to modify the method of Phillips in order to select a gene (e.g., a breeder marker) much less a polymorphic form of a gene by subtracting genomic DNA between sibling individuals. The method of Phillips only relies upon cDNAs as starting material in its subtraction strategy and Wigler teaches subtraction of genomic DNA between members of different families. Furthermore, neither of the references alone or combination teaches that the subtracted DNA should undergo an additional subtraction step against itself in order to eliminate intra-individual heterogeneity. Wigler when taken alone or in combination with Phillips, does not teach the inventive method as a whole (§2141.02 of the MPEP). Namely, the combined reference disclosures do not teach or suggest a novel object of the invention (step g) of claim 8) or even all of the steps that characterize the inventive method (steps c)- f) of claim 8).

The Examiner further relies upon Frazer for teaching that control of RDA experiments is important due to the numerous manipulations of templates where cross-contamination may be introduced through PCR amplification. In the simplest

of scenarios, Frazer discloses subtracting a tester sample from a driver sample, each generated from identical material, in order to ascertain the degree to which RDA is able to effectively deplete all sequences common to both pools. The purpose of the intra-individual subtraction step f) of claim 8 is quite unlike that of Frazer, namely, the method step is designed to eliminate **self-heterogeneity** from the overall inventive genome subtraction strategy of a forest tree plant. It is urged that the Examiner take note of the fact that genomic heterogeneity can occur within a single, given forest tree plant, and that eliminating any intra-individual background genomic variation is critical to the accuracy and sensitivity of the method designed to compare polymorphisms occurring between sibling-derived genomic DNA.

As a secondary reference Pinyopusarerk does not cure the deficiencies of the foregoing references alone or in combination, and therefore, Claim 13 is also patentable over Phillips, Wigler, Frazer and Pinyopusarerk.

Claims 10-12 and 15 are further limiting with respect to the patentable subject matter of Claim 8, and therefore, would not be considered obvious over Phillips, Wigler, Frazer and Pinyopusarerk.

Accordingly, the reference disclosures do not meet or satisfy the Examiner's *prima facia* case for obviousness of the instant claimed invention since the references do not teach or suggest the invention as a whole, the references do not teach or suggest that modifying either one of them would result in the claimed invention or even that one skilled in the art would have had a reasonable expectation of success in a obtaining an invention having all of the characteristics as presently claimed.

[redacted]

VI. Response to Rejection of Claim 13 under 35 U.S.C. 103(a)

Claim 13 is rejected under 35 U.S.C. 103(a) as being obvious over Phillips in view of Wigler, Frazer and Pinyopusarerk as applied to claims 8, 10-12 and 15, and further in view of Nainan et al.

The Examiner states that Nainan teaches a simple system to detect PCR products that has the sensitivity and specificity of nested PCR primer PCR which involves digoxigenin labeled PCR products. Therefore, according to the Examiner, it would have been *prima facie* obvious to label the cDNA of the method of Phillips with digoxigenin for the purposes of specifically detecting the cDNA.

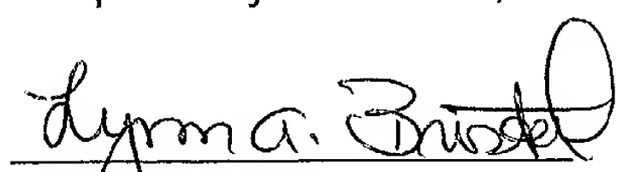
Applicants reiterate all of the arguments setting forth the patentability of Claims 8, 10-12 and 15 under section V with respect to Phillips, Wigler, Frazer and Pinyopusarerk. As a tertiary reference Nainan does not cure the deficiencies of the foregoing references alone or in combination, and therefore, Claim 13 is also patentable over Phillips, Wigler, Frazer, Pinyopusarerk and Nainan.

CONCLUSION

Applicants have demonstrated that each of the Examiner's cited references teach distinct and separate methods from not only each other, but in view of the instant claimed method. Accordingly, based on the foregoing amended claims and these arguments, Applicants submit that the Examiner's objections to and rejections of the claims under 35 U.S.C. §§103(a), the first and second paragraphs of 112 and 132, have been met and overcome. Applicants now request that the Examiner allow this application to pass to issuance.

In the event any fees are due in connection with this paper, please charge our Deposit Account No. 01-2300 referencing Client Matter No. 100021-09042.

Respectfully submitted,



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MARKED-UP COPY OF CLAIMS 8, 10, 12 AND 14 FOR USAN 09/444,388

8. (Twice Amended) A method for [obtaining a] identifying DNA [fragment for a breeding marker] for polymorphic forest tree plants, comprising the steps of:

a) selecting two sibling individuals of a forest tree plant having different phenotypes;

b) obtaining genomic DNA from the two individuals;

c) selecting DNA fragments by an inter-individual genome subtraction method using the genomic DNA from the two individuals;

d) providing [an RNA-derived labeled probe, wherein the probe is] a labeled cDNA probe derived from [of] all mRNA obtained from the two individuals, [and] wherein the cDNA is selected and amplified by oligonucleotide primers in a polymerase chain reaction [, wherein the primers are designed to hybridize to the mRNA for a plant gene related to the breeding marker];

e) fractionating the DNA fragments obtained by the genome subtraction of step c) and screening the DNA fragments with the RNA-derived labeled probe of step d);

f) [repeating steps c) to e) with] performing intra-individual subtraction with genomic DNA from one of the two individuals; and

[h)] g) comparing the DNA fragments of steps e) and f) to exclude the DNA fragments containing intra-individual polymorphisms [and to identify the DNA fragment for the breeding marker] and identifying the DNA fragments that are polymorphic between the individuals.

10. (Twice Amended) The method of claim 8, wherein the forest tree is [Acaia] Acacia.

12. (Twice Amended) The method of Claim 8, wherein the genome subtraction method is representation difference analysis [method].

14. (Twice Amended) The method of claim 8, wherein the oligonucleotide primers [comprise] consist of the sequences of SEQ ID NO: 1 and SEQ ID NO: 2.